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FURTHER INVESTIGATIONS OF THE SEROTONERGIC PROPERTIES OF THE IBOGAINE-INDUCED DISCRIMINATIVE STIMULUS

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Abstract

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1. 5-HT₃, 5-HT_{2C}, and 5-HT_{1A} receptor ligands were assessed in rats trained to discriminate ibogaine from water.
2. Significant ibogaine-appropriate responding was observed following treatment with the 5-HT_{2C} agonists MK-212 (79.6%) and mCPP (76.4%). This substitution was completely antagonized by metergoline, an agent with 5-HT_{2C} antagonist properties. However, metergoline was ineffective against ibogaine itself. This suggests that although ibogaine may act as an agonist at 5-HT_{2C} receptors, this interaction is not essential to its discriminative cue.
3. Neither the 5-HT₃ agonist, mCPBG (44.3%), nor the 5-HT₃ antagonist, ondansetron (48.9%) substituted for ibogaine. Likewise, the 5-HT_{1A} agonist 8-OH-DPAT (34.7%) and the 5-HT_{1A} antagonist WAY-100635 (30.1%) failed to substitute. Furthermore, WAY-100635 failed to antagonize the ibogaine cue.
4. Unlike 5-HT_{2C} receptors, 5-HT_{1A} and 5-HT₃ receptors do not appear to be involved in the ibogaine stimulus.

Keywords: drug discrimination, hallucinogens, ibogaine, serotonin receptors

Abbreviations: 1-(*m*-chlorophenyl)-biguanide (mCPBG), *m*-chlorophenylpiperazine (mCPP), 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), serotonin (5-HT).

Introduction

Because drug induced stimulus control has been shown to be of tremendous utility in the study of hallucinogens, this technique has been applied to the study of ibogaine, a hallucinogenic indole with reported antiaddictive effects. In the first report of ibogaine-induced stimulus control, Schechter and Gordon (1993) provided evidence that serotonergic interactions play a role in the ibogaine stimulus. Subsequent studies showed that while ibogaine acts as an agonist at 5-HT_{2A} receptors, this interaction

Gordon (1993) provided evidence that serotonergic interactions play a role in the ibogaine stimulus. Subsequent studies showed that while ibogaine acts as an agonist at 5-HT_{2A} receptors, this interaction is not essential for ibogaine-induced stimulus control. Specifically, the (partial) generalization of ibogaine to both LSD and (-)-2,5-dimethoxy-4-methylamphetamine (DOM) was blocked by 5-HT_{2A} antagonists but ibogaine itself was not (Helsley *et al.*, 1998a). It is possible that ibogaine's interactions with serotonergic receptors are not limited to those of the 5-HT_{2A} subtype. This is supported by the fact that serotonin releaser, fenfluramine, produces greater than 70% ibogaine-appropriate responding in ibogaine-trained rats (Schechter and Gordon, 1993).

The present study was designed to investigate possible contributions by 5-HT_{1A}, 5-HT_{2C}, and 5-HT₃ receptors to the ibogaine stimulus. Of these, the 5-HT₃ receptor appears most likely to be involved in the effects of ibogaine. For example, the structural similarity between ibogaine and certain 5-HT₃ antagonists is striking. This, taken together with the fact that 5-HT₃ antagonists may be effective in the treatment of certain substance abuse disorders (Grant, 1995), suggests that ibogaine may mediate some of its effects via antagonist interactions with these receptors. Indeed, with respect to *in vitro* binding studies, of all the 5-HT receptors examined, ibogaine has its highest affinity for 5-HT₃ receptors [3.9 μ M] (Sweetnam *et al.*, 1995).

5-HT_{2C} and 5-HT_{1A} receptors may also mediate some of ibogaine's effects. A role for 5-HT_{2C} receptors is plausible because these receptors play a modulatory role in hallucinogenesis (Fiorella *et al.*, 1995 a,b,c). In addition certain structural congeners of ibogaine have appreciable affinity for this receptor (Dr. David Repke, personal communication). Our interest in ibogaine's effects at the 5-HT_{1A} receptor was prompted by the observation that stimulus control induced by the hallucinogen 5-methoxy-N,N-dimethyltryptamine, involves a predominant 5-HT_{1A} component (Spencer *et al.*, 1987). Because of the structural similarity between this agent and ibogaine, it is possible that 5-HT_{1A} receptors are integral to ibogaine's mechanism of action.

Methods

Animals

Male Fischer 344 rats were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN). They were housed in pairs in a room with windows, allowing for a natural light-dark cycle. Subjects were fed following experimental sessions. Caloric intake was controlled to yield a mean bodyweight of about 250 grams. Water was available at all times in the home cage.

Drugs

Ibogaine HCl was provided by the National Institute on Drug Abuse. The following agents were purchased from commercial sources: mCPP (Aldrich Chemical, Milwaukee, Wis.); mCPBG HCl, and 8-OH-DPAT HBr (Research Biochemicals, Natick, MA). The following compounds were generously provided by the indicated organizations: WAY-100635 (Wyeth-Ayerst, Princeton, NJ); ondansetron (Glaxo Research, Greenford, Middlessex, UK); MK-212 (Merck Research Laboratories, West Point, PA); metergoline (Farmaceutica Italia, Italy). All drugs were dissolved in water with the exception of metergoline which was dissolved in water with a few drops of 8.5% lactic acid. Solutions were injected i.p. in a volume of 1.0 ml/kg bodyweight with the exception of WAY-100635 which was administered via s.c. injection.

Apparatus

Six small animal test chambers (Coulbourn Instruments Model E10-10) housed in larger light-proof, sound insulated boxes were used for all experiments. Each box had a house light and exhaust fan. The chamber contained two levers mounted on opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Ibogaine-Induced Stimulus Control

24 subjects were trained to discriminate ibogaine (10.0 mg/kg, 60 minute pretreatment time, intraperitoneal injection) from water as previously described (Fiorella et al., 1995d; Helsley et al., 1997). A fixed ratio 10 (FR10) schedule of reinforcement was employed. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. After stimulus control was established with ibogaine, tests were conducted once per week in each animal so long as performance did not fall below the criterion level of 83% correct responding in any one of the previous three training sessions.

Test Procedure

Half of the test sessions were conducted the day after vehicle training sessions with the remainder following ibogaine training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted prior to lever selection, that is, prior to the emission of 10 responses on either lever,

by the elapsed time. The data for subjects failing to emit 10 responses within the constraints of the ten minute test session were not considered in the calculation of percent drug-appropriate responding. Pretreatment times were 60 minutes for ibogaine, 30 minutes for ondansetron (Fiorella *et al.*, 1995e) and WAY-100635 (Fletcher *et al.*, 1996), 25 minutes for mCPP and MK 212 (Fiorella *et al.*, 1995b) and 15 min for 8-OH-DPAT (Rabin and Winter, 1993) and mCPBG (De La Garza *et al.*, 1996). Metergoline was given 15 min prior to the agent against which it was tested (Callahan and Cunningham, 1994).

Data Analysis

The criteria for generalization and antagonism are similar to those used in previous reports (Winter and Rabin 1992). Complete generalization/no antagonism is said to be present when [i] a mean of 83% or more of all test responses are on the drug-appropriate lever, [ii] there is no statistically significant difference between training-drug and test-drug response distributions, and [iii] there is a statistically significant difference between test-drug and vehicle-control response distributions. An intermediate degree of generalization/antagonism is defined as being present when mean response distributions following a test-drug show a statistically significant difference from distributions following both training conditions with $\geq 50\%$ drug-appropriate responding. Finally, when response distributions following a test-drug are not significantly different from vehicle-control response distributions, no generalization/full antagonism is assumed.

Comparisons of data are by means of individual applications of Wilcoxon's signed ranks test. Thus, data obtained with a given drug at a given dose are compared with the immediately preceding training sessions for vehicle and training-drug, respectively. Differences are considered to be significant if they would be expected to arise by random sampling alone with a probability < 0.05 . The substitution produced by both mCPP and MK-212 alone was compared to that produced by these agents in the presence of metergoline using the Mann-Whitney Rank Sum test with $p < 0.05$ considered significant.

Results

Dose-response Relationships for 5-HT_{2C}, 5-HT₃, and 5-HT_{1A} Receptor Ligands.

Figure 1 shows the partial generalization of ibogaine to the 5-HT_{2C} receptor agonists mCPP (76.4%) and MK-212 (79.6%) as well as the lack of generalization to the 5-HT_{1A} ligands 8-OH-DPAT and WAY-100635 and the 5-HT₃ receptor ligands mCPBG and ondansetron.

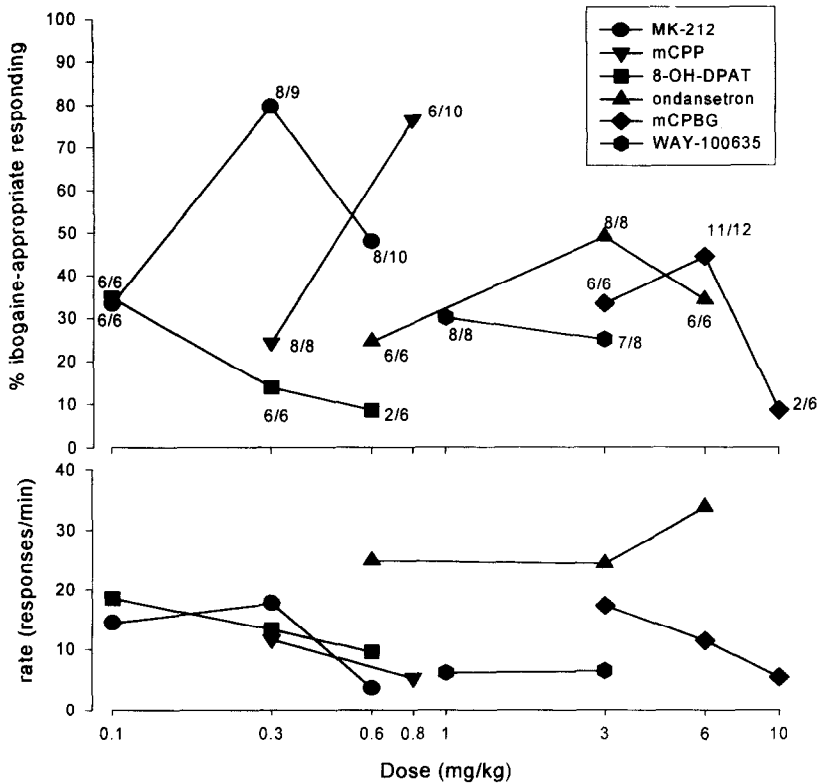


Fig 1. Dose-response relationships for 5-HT_{2C}, 5-HT₃, and 5-HT_{1A} receptor ligands in rats trained with 10.0 mg/kg ibogaine as a discriminative stimulus. MK-212 and mCPP were administered ip 25 min pre-session. Ondansetron and WAY-100635 were given 30 min pre-session (i.p. and s.c., respectively) while 8-OH-DPAT and mCPBG were both administered i.p. 15 min pre-session. The ratio adjacent to each of the points represents the number of subjects completing the test session over the number of subjects participating in each test session. Ordinate: Percent ibogaine-appropriate responding. Abscissa: Dose of test agent.

Tests of Antagonism in Ibogaine-Trained Rats.

In keeping with the hypothesis that the ibogaine appropriate responding produced by both MK-212 and mCPP is mediated by 5-HT_{2C} receptors, metergoline, an antagonist at these receptors (Callahan and Cunningham, 1994) completely blocked the substitution produced by both agents. However

Furthermore, a previous report described a similar lack of ibogaine antagonism with mesulergine (Helsley *et al.*, 1998a), another agent with 5-HT_{2C} antagonist properties (Fiorella *et al.*, 1995d). To further investigate ibogaine's actions at 5-HT_{1A} receptors, the selective 5-HT_{1A} antagonist, WAY-100635 (Forster *et al.*, 1995; Fletcher *et al.*, 1996) was tested in combination with ibogaine. No antagonism was observed. The present results are summarized in Table 1.

Table 1
5-HT Receptor Antagonists in Ibogaine-trained Rats

Drug Treatment	% Ibogaine-Appropriate Responses	Rate (Responses/min)	n/N
ibogaine	94.0	14.3	10/10
ibogaine (10 mg/kg) + mesulergine (20 mg/kg) [#]	98.5	9.7	6/6
ibogaine (10 mg/kg) + metergoline (1.0 mg/kg)	100	36.4	4/4
MK-212 (0.3 mg/kg) + metergoline (1.0 mg/kg)**	10.0 [79.6]	16.1	7/8
mCPP (0.8 mg/kg) + metergoline (1.0 mg/kg)**	23.8 [76.4]	13.0	6/8
ibogaine (10 mg/kg) + WAY-100635 (0.3 mg/kg)	84.3%	11.1	8/8

The ratio n/N represents the number of animals responding (n) out of the number of animals tested (N). The % ibogaine-appropriate responding produced by both mCPP and MK-212 alone is enclosed in brackets. Treatment sessions were compared to immediately preceding ibogaine training sessions using Wilcoxon's signed ranks test. * reflects significant differences from the ibogaine-treatment condition ($p < 0.05$). ** reflects significant differences between the drug alone and the drug + antagonist conditions as determined by Mann-Whitney Rank Sum test.

[#] refers to previously reported data (Helsley *et al.*, 1998a).

Discussion

The present study provides evidence for the involvement of 5-HT_{2C} receptors in the stimulus effects of ibogaine. These findings, taken together with the results of a previous study which provided evidence that 5-HT_{2A} receptors are involved in the ibogaine stimulus (Helsley *et al.*, 1998a), confirm the hypothesis that ibogaine acts at least partly via serotonergic mechanisms (Sloviter *et al.*, 1980). The fact that the ibogaine cue is not antagonized by serotonergic antagonists suggests that these interactions are not essential to the ibogaine-trained stimulus. This in no way means they are insignificant. Instead these findings suggest that the ibogaine stimulus is complex in nature, being mediated via interactions with multiple receptors. This hypothesis fits well with both our observations and the findings of others. For example, in their comprehensive assessment of the receptor binding

mediated via interactions with multiple receptors. This hypothesis fits well with both our observations and the findings of others. For example, in their comprehensive assessment of the receptor binding profile of ibogaine, Sweetnam and colleagues (1995) proposed that ibogaine may produce its unique effects via multiple mechanisms. Correspondingly, in a recent report Ali et al. (1996) wrote "Ibogaine binds to multiple sites in the nervous system, and coactivation of multiple transmitter systems probably accounts for ibogaine's diverse actions". Our findings certainly support the hypothesis that ibogaine interacts with a variety of serotonin receptor subtypes. In addition, another recent report suggests that opiate receptors as well as σ_2 receptors are also involved in the ibogaine stimulus (Helsley et al., 1998b).

The fact that both MK 212 and mCPP both produced significant ibogaine-appropriate responding strongly argues for a role of 5-HT_{2C} receptor in the ibogaine stimulus. Even though both of the aforementioned agents may be non-specific, as mentioned above they do share 5-HT_{2C} agonist activity in common. The possibility that 5-HT_{2A} receptor agonism may mediate the ibogaine-like effects of these agents seems especially unlikely as mCPP has been shown to act as an antagonist at 5-HT_{2A} receptors (Conn and Sanders-Bush, 1987).

Ibogaine's interactions with 5-HT_{2C} receptors may be involved in its antiaddictive effects. A potential role for these receptors in addiction phenomena comes from a study by Wang et al. (1995) in which the ability of fenfluramine to reduce heroin self administration in rats was reversed following pretreatment with metergoline. Furthermore ibogaine's interactions with these receptors may be involved in its hallucinogenic effects as previous studies offer evidence that 5-HT_{2C} receptors play a modulatory role in the stimulus effects of hallucinogens (Fiorella et al., 1995a,b,c). This raises the question as to whether ibogaine's hallucinogenic properties are integral to its antiaddictive effects. The answer to this question lies well beyond the scope of the present study but a recent review article calls for a re-evaluation of hallucinogens as addiction therapies (Halpern, 1996).

The lack of substitution observed with 5-HT₃ receptor ligands is somewhat surprising in light of the fact that antagonists at this receptor both resemble ibogaine structurally and have been observed to be efficacious in the treatment of ethanol abuse (Sellers et al., 1994). Nevertheless, it is possible that ibogaine produces its reported antiaddictive effects (Sheppard, 1994) via interactions with these receptors but the present study does not support a role for this receptor subtype in the ibogaine discriminative stimulus.

Conclusion

The present study offers evidence that 5-HT_{2C} receptors play a role in the ibogaine discriminative stimulus. In contrast, the present study argues against involvement of 5-HT_{1A} and 5-HT₃ receptors. These findings, taken together with those of previous studies (Sweetnam *et al.*, 1995; Helsley *et al.*, 1998a,b) suggests that ibogaine interacts with multiple receptor subtypes.

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